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HYPERBRANCHED POLYAMIDOAMINE

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The present invention relates to hyperbranched polymers, and more particularly to certain novel hyperbranched polymers, to novel methods for their production, to compositions of hyperbranched polymers with useful agents, and to the use of hyperbranched polymers in *inter alia* gene transfection.

Dendrimers and hyperbranched polymers are attracting increasing levels of interest in various fields of research. The molecules of a dendrimer are characterised by highly regular and radially symmetrical branching about a core atom. The degree of branching is 100% and dendrimers exhibit a precisely defined molecular weight. The synthesis of dendrimers using iterative synthetic procedures is well established. For example, US-A-4568737, US-A-4587329, US-A-4558120, US-A-4507466 and US-A-4435548 describe the preparation of symmetrical (*ie* NR_3) PAMAM dendrimers by performing on a core moiety (such as ammonia) successive Michael additions and amidation using excess reagents or successive amidation and alkylation steps.

Polymers obtained from the statistical polymerisation of AB_x monomers by means of condensation or addition procedures are referred to as hyperbranched polymers. These structures are primarily formed via polycondensation of AB_x monomers which introduce the branching but do not allow gelation. In these polymers, the branching is controlled by statistics and reaches for an AB_2 monomer only about 50% compared to the 100% branching of a perfectly branched dendrimer. In addition, no control over size and structure is given and the polymers exhibit a broad molar mass distribution.

Generally hyperbranched polymers have an irregular branched structure, are not generally characterised by MS or NMR and (unlike dendrimers) exhibit a broad GPC trace. Hyperbranched polymers are characterised by the presence of successive units of a generic structural repeating unit (SRU) having a connectivity of more than two. In addition, hyperbranched polymers have a multitude of end groups (hereinafter "terminal units") and can also include bridging SRUs with a connectivity of two.

Gene therapy is a new and potentially revolutionary technology which could dramatically restructure the way in which certain diseases are treated and possibly provide cures for currently untreatable genetic diseases. Advances in this technology are being seriously hampered by the lack of effective, safe and cheap transfection agents capable of delivering therapeutic genes to the patient. Moreover, laboratory research is suffering due to the lack of efficient and versatile transfection agents required for preliminary investigations into new therapies.

As used herein, a vector is a compound which can deliver DNA into cell lines. The present market for gene transfection is dominated by viral (*ie* retroviral or adenoviral) or non-viral vectors such as synthetic cationic liposomes (lipoplexes). Viral vectors are very efficient at delivering DNA into cells but have several drawbacks including the need for specialist handling conditions, immunogenicity and potentially serious side effects (such as recombination of viral DNA with host DNA). The leading non-viral vector is LIPOFECTAMINE^{R}. The main disadvantage of this lipid based vector is that it

is toxic and has limited use *in vivo* being a dynamic structure which can easily fall apart below a certain critical concentration. Several attempts have been made to modify the structure of the lipid to make it less toxic (for example by adding biocompatible molecules). To date, none of these attempts have been successful and toxicity is still the major drawback.

Other non-viral vectors available on the market include polyamidoamine (PAMAM) dendrimers and several other synthetic polymers (polyplexes) which are mostly linear in structure or possess very limited branching (such as polyethyleneimine, polylysine and several other amino acid derived polymers). PAMAM dendrimers may be used intact or partially degraded (often being referred to as activated dendrimers (*eg* SUPERFECT^{R})). Generally these agents require activation (*eg* by thermal degradation).

Lim *et al*, J Am Chem Soc, 2001, 123, 2460-2461 discloses the use of a certain hyperbranched polyaminoester for gene transfection. This hyperbranched polyaminoester was prepared by first synthesising a monomer by Michael addition of ethanolamine with methyl acrylate followed by bulk polymerisation in the presence of a catalyst. In order to enable the polyaminoester to condense negatively charged DNA, the surface of the polymer was functionalised by converting methyl ester groups into amino groups in two further steps. The degree of conversion was less than 80%. Lim *et al* reported that the surface modified polyaminoester could transfect DNA and exhibited low toxicity. However, several synthetic steps are required to synthesise the polyaminoester and the transfection efficiency is low.

In one embodiment the present invention provides new amidoamine polymers and a new method for their preparation which can involve fewer steps than hitherto. In a further embodiment the invention provides new hyperbranched polymers useful in *inter alia* gene transfection which may be both efficient and safe for use in clinical applications.

According to a first aspect of the invention, there is provided a hyperbranched amidoamine polymer comprising [A] a first structural repeating unit having a connectivity of three consisting of a nitrogen core linked to a first amidoamine unit, a second amidoamine unit and a third amidoamine unit, [B] a second structural repeating unit consisting of a nitrogen core linked to a first amidoamine unit and a second amidoamine unit and having a connectivity of two, and [C] terminal units of which a major portion comprises amine groups or a functional derivative thereof, and a minor portion comprises carboxylic acid or related groups or a functional derivative thereof.

Hyperbranched amidoamine polymers of this aspect of the invention have a structure which comprises SRUs with a connectivity of three, which give rise to the hyperbranched structure, SRUs with a connectivity of two, which give rise to chain extension, and terminal units. The hyperbranched amidoamine polymer structure can be derived from the condensation of a single tri-functional monomer of appropriate configuration, or from the condensation of two or more monomers. Preferably the polymer structure is derived substantially from the

condensation of a single tri-functional monomer. In such a polymer structure, an SRU with a connectivity of three is formed when each of the three functional groups of the monomer is connected to or forms part of a further branch. Similarly, an SRU with a connectivity of two is formed when two of the three functional groups are connected to or form part of a branch. A terminal unit can be formed in three ways. Firstly a terminal unit can simply comprise a functional group at the end of a branch. Secondly it can be formed by the third functional group of an SRU with a connectivity of two. Thirdly it can be formed by connection of a terminal group to the said third functional group or to a functional group at the end of a branch.

The ratio of tri-connective SRUs to di-connective SRUs to terminal units in the polymer is preferably in the range of 1:10:20 to 1:2: 2.5.

The first, second, and third amidoamine units of the first SRU and the first and second amidoamine units of the second SRU can each independently be the same or different as will be explained hereinafter.

In a first preferred aspect the present invention provides a hyperbranched amidoamine polymer whose molecules are characterised by a nitrogen core linked to:
a first irregularly branched amidoamine structural unit terminating in an amine group or a functional derivative thereof;
a second irregularly branched amidoamine structural unit terminating in an amine group or a functional derivative thereof; and

a third irregularly branched amidoamine unit terminating in a carboxylic acid or related group or a functional derivative thereof.

The molecules of the preferred hyperbranched amidoamine polymers of the invention are collectively characterised by the irregularity of the branching in the first, second and third amidoamine units and it is this which distinguishes them structurally over dendrimers and may account for their more favourable properties. An irregularly branched amidoamine structural unit of this aspect of the invention is one which lacks a centre of symmetry.

The hyperbranched amidoamine polymers of the invention have potentially extensive utility in numerous systems. Broadly speaking, they offer a multiplicity of functional groups together with a large surface area and internal volume and as such may be widely exploited as carriers, supports or substrates. The hyperbranched amidoamine polymers of the invention are typically stable for lengthy periods (*eg* one year or more) and may be at least as effective in gene transfection as the market leaders. They can be structurally more flexible than dendrimers and may have the advantage of being water soluble.

Preferably the hyperbranched amidoamine polymers can have a theoretical degree of branching up to 50%, particularly preferably up to 67%, more preferably up to 75%, most preferably up to 80%.

Preferably each of the first, second and third irregularly branched amidoamine units, which may be the

same or different, includes consecutive, irregularly branched amidoamine moieties each having two or more (preferably two or three) amido groups.

Preferably the amine group or functional derivative thereof (in which the first and second irregularly branched amidoamine unit terminates) is a primary amine group or a functional derivative thereof. The functional derivative of the amine group may be chosen to suit the desired function of the hyperbranched amidoamine polymer. For example, the functional derivative may be a secondary, tertiary or quaternary amine group, an aromatic or aliphatic amide group, a cyano group, a sulphur containing group (*eg* a thioamide group), a cross-linking group (*eg* for cross-linking to other polymers or oligomers), an anilino group or an acyclic polynitrogen group (*eg* a guanidino, biguanidino, triguanidino or ureido group).

Preferably the functional derivative is an amine group substituted with one, two or three C_{1-6}-alkyl groups (*eg* methyl groups) or with an *N,N*-substituted amidoamine group.

Preferably the functional derivative is a quaternary amine group which is cationic and can be advantageously exploited for binding DNA in gene transfection.

Preferably the related group of the carboxylic acid is selected from the group consisting of a salt, ester, anhydride, acid halide (*eg* chloride), acyl, amide, imide, nitrile, aldehyde and hydrazide. The functional derivative may be a carboxyl protecting or blocking group

or a group chosen to suit the desired function of the hyperbranched amidoamine polymer. Preferably the third irregularly branched amidoamine unit terminates in a carboxylic acid group or a functional derivative thereof.

Preferably the molecules of the hyperbranched amidoamine polymer are characterised by formula I:

$$\text{(I)} \qquad \text{T-R}^3\text{-CO-Y-N}\begin{matrix} \diagup \text{R}^1\text{T}^1 \\ \diagdown \text{R}^2 \end{matrix}$$

wherein:

Y is a divalent bridging group;

T together with a terminal CO group of R^3 to which it is bound is a carboxylic acid or related group or a functional derivative thereof;

T^1 together with a terminal nitrogen atom of R^1 to which it is bound is an amine group or functional derivative thereof;

R^1 is an amidoamine unit of formula II:

$$\text{(II)} \qquad \text{-Y}'\text{-CO-NH-X-N}\begin{matrix} \diagup \text{R}^4 \\ \diagdown \text{R}^5 \end{matrix}$$

wherein:

each of X and Y′ which may be the same or different is a divalent bridging group;

R^4 is either

(a) n consecutive amidoamine moieties of formula III:

(III) $-(Y''-CO-NH-X'-NH)_s-CO-Y-NR^2-Y'-CO-NH-X-NR^5-$

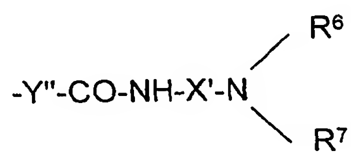
wherein:

s is 0 or 1;

n is a number greater than 0;

each of X′ and Y′′ which may be the same or different is a divalent bridging group or

(b) an amidoamine unit of formula IV

(IV) 

wherein:

R^6 is either

(a) m consecutive amidoamine moieties of formula V:

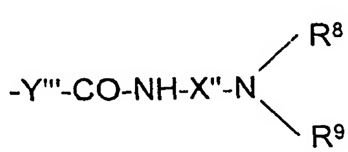
(V) $-Y'''-CO-NH-X''-NH-CO-Y-NR^2-Y'-CO-NH-X-NR^5-Y''-CO-NH-X'-NR^7-$

wherein:

m is a number greater than 0;

each of X′′ and Y′′′ which may be the same or different is a divalent bridging group) or

(b) an amidoamine unit of formula VI

(IV) 

wherein:

R^8 is x consecutive amidoamine moieties of formula VII:

(VII)

$-Y''''-CO-NH-X'''-NH-CO-Y-NR^2-Y'-CO-NH-X-NR^5-Y''-CO-NH-X'-NR^7-Y'''-CO-NH-X''-NR^9-$

wherein:

x is a number greater than 0;

each of X''' and Y'''' which may be the same or different is a divalent bridging group; and

R^9 is R^1 T^1 or is a group as hereinbefore defined for R^8T^1 wherein T^1 together with a terminal nitrogen atom of R^8 to which it is bound is an amine group or functional derivative thereof); and

R^7 is R^1 T^1 or is a group as hereinbefore defined for R^6T^1 wherein T^1 together with a terminal nitrogen atom of R^6 to which it is bound is an amine group or functional derivative thereof); and

R^5 is R^1 T^1 or a group as hereinbefore defined for R^4T^1 wherein T^1 together with a terminal nitrogen atom of R^4 to which it is bound is an amine group or functional derivative thereof); and

R^2 is as hereinbefore defined for R^1T^1; and

R^3 is either

(a) p consecutive amidoamine moieties of formula VIII:

(VIII) **$-CO-Y-NR^2-Y'-CO-NH-X-NR^5-(Y''-CO-NH-X'-NH)_s-$**

wherein:

p is a number of more than zero

or (b) q consecutive amidoamine moieties of formula IX:

(IX) $-CO-Y-NR^2-Y'-CO-NH-X-NR^5-Y''-CO-NH-X'-NR^7-Y'''-CO-NH-X''-NH-$

wherein:

q is a number greater than 0

or (c) y consecutive amidoamine moieties of formula X

(X) $-CO-Y-NR^2-Y'-CO-NH-X-NR^5-Y''-CO-NH-X'-NR^7-Y'''-CO-NH-X''-NR^9-Y''''-CO-NH-X'''-NH-$

wherein:

y is a number greater than 0).

For the avoidance of doubt, R^1 T^1 may be the same as or different from R^2 (but preferably is the same), R^4 T^1 may be the same as or different from R^5 (but preferably is the same), R^6 T^1 may be the same as or different from R^7 (but preferably is the same) and R^8 T^1 may be the same as or different from R^9 (but preferably is the same).

In a first preferred embodiment, R^4 is option (a) and s is 0.

In a second preferred embodiment, R^4 is option (a) and s is 1.

In a third preferred embodiment, R^4 is option (b) and R^6 is option (a).

In a fourth preferred embodiment, R^4 is option (b) and R^6 is option (b).

The average molecular weight molecule is represented by the aforementioned formula I in which n+p or m+q or x+y is in the range 1 to 20.

Each of Y, Y′, Y′′, Y′′′, Y′′′′, X, X′, X′′ and X′′′ which may be the same or different may be a cyclic (*eg* monocyclic) hydrocarbon (*eg* aromatic hydrocarbon) bridging group, an acyclic heteroatomic bridging group, a heterocyclic (*eg* heteroaromatic) bridging group or an acyclic hydrocarbon bridging group (which itself is optionally interrupted by or terminates in one or more of a cyclic (*eg* monocyclic) hydrocarbon (*eg* aromatic hydrocarbon) group, an acyclic heteroatomic group, a heterocyclic (*eg* heteroaromatic) group or amide group). The bridging groups should be chosen so as not to interfere with polymerisation.

By way of example, each of Y, Y′, Y′′, Y′′′, Y′′′′, X, X′, X′′ and X′′′ which may be the same or different may be a C_{1-12}-alkylene or C_{1-12}-alkenylene bridging group optionally interrupted by or terminating in an oxygen atom, one, two or three optionally (but preferably) substituted nitrogen atoms, a cyclic (*eg* monocyclic) hydrocarbon (*eg* aromatic hydrocarbon) group, a heterocyclic (*eg* heteroaromatic) group or an amide group.

Preferably each of Y, Y′, Y′′, Y′′′, Y′′′′, X, X′, X′′ and X′′′ which may be the same or different is a C_{1-6}-alkylene, particularly preferably is a C_{1-4}-alkylene bridging group (*eg* ethylene). Preferably each of Y, Y′, Y′′, Y′′′, Y′′′′, X, X′, X′′ and X′′′ is ethylene.

Preferably T is selected from the group consisting of Cl, O-CO-R^{10}, NHR^{12}, =NH, /N, H, OR^{11} and OMet (wherein each of R^{10} and R^{11} which may be the same or different is hydrogen or an optionally substituted C_{1-12}-alkyl group (*eg* C_{1-6}-alkyl group); R^{12} is hydrogen, an optionally substituted C_{1-12}-alkyl group (*eg* C_{1-6}-alkyl group) or NHR^{10}; and Met is a metal (*eg* an alkali or alkaline earth metal)). Preferably T is hydroxyl.

Preferably T^1 is selected from the group consisting of hydrogen and *N*-substituents rendering the nitrogen to which they are bound a functional derivative of amine (*eg* one or two C_{1-6}-alkyl (eg methyl) groups).

In a preferred embodiment, the hyperbranched amidoamine polymer is obtainable by polymeric condensation of a compound in which a nitrogen core is linked to:
a first amidoamine, (*N,N*-diamidoamine)amidoamine, *N,N*-di(*N,N*-diamidoamine)amidoamine or *N,N*-di(*N,N*-di(*N,N*-diamidoamine)amidoamine)amidoamine unit terminating in an amine group;
a second amidoamine, (*N,N*-diamidoamine)amidoamine, *N,N*-di(*N,N*-diamidoamine)amidoamine or *N,N*-di(*N,N*-di(*N,N*-diamidoamine)amidoamine)amidoamine unit terminating in an amine group; and
a third unit terminating in a carboxylic acid or related group.

In a further aspect, the present invention seeks to provide an improved process for preparing hyperbranched amidoamine polymers which is advantageously carried out in a single step. More particularly, the process relates to a single step synthesis of a hyperbranched amidoamine

polymer with a broad molecular weight distribution by polycondensation without the need for additional functionalisation steps such as thermal degradation.

Viewed from a still further aspect the present invention provides a process for preparing a hyperbranched amidoamine polymer comprising:

(A) inducing polymeric condensation of a compound in which a nitrogen core is

linked to:

a first amidoamine, (*N*-amidoamine)amidoamine, *N*-(*N*-amidoamine)amidoamine or *N*-(*N*-(*N*-amidoamine)amidoamine)amidoamine unit terminating in an amine group;

a second amidoamine, (*N*-amidoamine)amidoamine, *N*-(*N*-amidoamine)amidoamine or *N*-(*N*-(*N*-amidoamine)amidoamine)amidoamine unit terminating in an amine group; and

a third unit terminating in a carboxylic acid or related group.

In a preferred embodiment of the process, the nitrogen core is linked to

a first amidoamine, (*N*,*N*-diamidoamine)amidoamine, *N*,*N*-di(*N*,*N*-diamidoamine)amidoamine or *N*,*N*-di(*N*,*N*-di(*N*,*N*-diamidoamine)amidoamine)amidoamine unit terminating in an amine group;

a second amidoamine, (*N*,*N*-diamidoamine)amidoamine, *N*,*N*-di(*N*,*N*-diamidoamine)amidoamine or *N*,*N*-di(*N*,*N*-di(*N*,*N*-diamidoamine)amidoamine)amidoamine unit terminating in an amine group; and

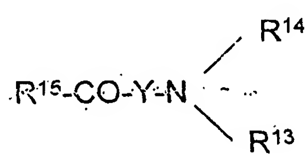
a third unit terminating in a carboxylic acid or related group.

The process advantageously leads to short manufacturing times and requires non-specialist equipment (*eg* standard laboratory equipment) so is uncostly.

Preferably the terminal amine group is a primary amine group.

Preferably the related group of the carboxylic acid is selected from the group consisting of a salt, ester, anhydride, acid halide (*eg* chloride), acyl, amide, imide, nitrile, aldehyde and hydrazide. Preferably the third unit terminates in a carboxylic acid group.

In a preferred embodiment, the compound is of formula XI

(XI) 

wherein:
Y is as hereinbefore defined;
R^{15} is as hereinbefore defined for group T;
each of R^{13} and R^{14} which may be the same or different is a group $-Y'-CO-NH-X-NH_2$, $-Y'-CO-NH-X-NR^{16}(Y''-CO-NH-X'-NR^{17}R^{18})$ (wherein R^{16} is hydrogen or $-Y''-CO-NH-X'-NR^{17}R^{18}$; each of R^{17} and R^{18} which may be the same or different is hydrogen or $-Y'''-CO-NH-X''-NR^{19}R^{20}$ (wherein each of R^{19} and R^{20} which may be the same or different is hydrogen or $-Y''''-CO-NH-X'''-NH_2$); and

Y′, X, X′, X′′, X′′′, Y′′′, Y′′′′ and Y′′ are as hereinbefore defined).

Preferably R^{15} is hydroxyl.

In a first preferred embodiment, R^{13} and R^{14} are both the group -Y′-CO-NH-X-NH_2 (an AB^2-type monomer).

In a second preferred embodiment, R^{13} and R^{14} are both the group -Y′-CO-NH-X-N-(Y′′-CO-NH-X′-NH_2)$_2$ (an AB^4-type monomer).

In a third preferred embodiment, R^{13} and R^{14} are both the group -Y′-CO-NH-X-N-(Y′′-CO-NH-X′-N(Y′′′-CO-NH-X′′-NH_2)$_2$)$_2$ (an AB^8-type monomer).

In a fourth preferred embodiment, R^{13} and R^{14} are both the group -Y′-CO-NH-X-N-(Y′′-CO-NH-X′-N(Y′′′-CO-NH-X′′-N(Y′′′′-CO-NH-X′′′-NH_2)$_2$)$_2$)$_2$ (an AB^{16}-type monomer).

Particularly preferably the compound of formula XI is an AB^2-type or AB^4-type monomer.

In the first preferred embodiment, step (A) is preferably preceded by:

(A0) reacting a diamine of formula NH_2-X-NH_2 with a compound of formula XII:

$$\text{(XII)}\qquad R^{15}\text{-CO-Y-N}\begin{cases}\text{Y'-CO-}R^{22}\\ \text{Y'-CO-}R^{21}\end{cases}$$

(wherein R^{21} and R^{22} which may be the same or different are as hereinbefore defined for group T and Y′, R^{15} and Y are as hereinbefore defined). Preferably each of R^{21} and R^{22} which may be the same or different (but preferably are

the same) is an OC_{1-6}-alkyl group, particularly preferably OMe.

In the first preferred embodiment, step (A0) is preferably preceded by:

(A00) reacting a compound of formula XIII:

$$\textbf{(XIII)} \qquad R^{15}\text{-CO-Y-NH}_2$$

(wherein Y and R^{15} are as hereinbefore defined) with a Michael addition reagent.

In the second preferred embodiment, step (A) is preferably preceded by:

(A′0) reacting a diamine of formula NH_2-X′-NH_2 with a compound of formula XIV:

$$\text{(XIV)} \qquad R^{15}\text{-CO-Y-N}\begin{cases}\text{Y'-CO-NH-X-N(Y''-CO-}R^{24})_2\\ \text{Y'-CO-NH-X-N(Y''-CO-}R^{23})_2\end{cases}$$

(wherein R^{23} and R^{24} which may be the same or different are as hereinbefore defined for group T and X, X′, Y, Y′ and Y′′ are as hereinbefore defined). Preferably each of R^{23} and R^{24} which may be the same or different (but preferably are the same) is an OC_{1-6}-alkyl group, particularly preferably OMe.

In the second preferred embodiment, step (A′0) is preferably preceded by:

(A′00) reacting a compound of formula XV:

(XV) $R^{15}\text{-CO-Y-N}\begin{cases} R^{25} \\ R^{26} \end{cases}$

(wherein Y and R^{15} are as hereinbefore defined; and each of R^{25} and R^{26} which may be the same or different is a group $-Y'-CO-NH-X-NH_2$ wherein X and Y′ are as hereinbefore defined) with a Michael addition reagent.

The compound of formula XV may itself be prepared from a compound of formula XII by step (A0) as hereinbefore defined.

In the third preferred embodiment, step (A) is preferably preceded by:

(A′′0) reacting a diamine of formula $NH_2-X''-NH_2$ with a compound of formula XVI:

(XVI) $R^{15}\text{-CO-Y-N}\begin{cases} \text{Y'-CO-NH-X-N-Y''-CO-NH-X'-N-(Y'''-CO-}R^{28})_2 \\ \text{Y'-CO-NH-X-N-Y''-CO-NH-X'-N-(Y'''-CO-}R^{27})_2 \end{cases}$

(wherein R^{27} and R^{28} which may be the same or different are as hereinbefore defined for group T and X, X′, X′′, Y, Y′, Y′′ and Y′′′ are as hereinbefore defined). Preferably each of R^{27} and R^{28} which may be the same or different (but preferably are the same) is an OC_{1-6}-alkyl group, particularly preferably OMe.

In the third preferred embodiment, step (A′′0) is preferably preceded by:

(A′′00) reacting a compound of formula XVII:

(XVII) R^{15}-CO-Y-N
╱ R^{29}
╲ R^{30}

(wherein Y and R^{15} are as hereinbefore defined; and each of R^{29} and R^{30} which may be the same or different is a group Y′-CO-NH-X-N-Y′′-CO-NH-X′-NH_2 wherein X, X′, Y′ and Y′′ are as hereinbefore defined) with a Michael addition reagent.

The compound of formula XVII may itself be prepared from a compound of formula XIV by step (A′0) as hereinbefore defined.

In the fourth preferred embodiment, step (A) is preferably preceded by:

(A′′′0) reacting a diamine of formula NH_2-X′′′-NH_2 with a compound of formula XVIII:

(XVIII)

R^{15}-CO-Y-N
╱ Y'-CO-NH-X-N-Y"-CO-NH-X'-N-Y'''-CO-NH-X"-N-(Y''''-CO-R^{31})$_2$
╲ Y'-CO-NH-X-N-Y"-CO-NH-X'-N-Y-'''-CO-NH-X"-N-(Y''''-CO-R^{32})^2

(wherein R^{31} and R^{32} which may be the same or different are as hereinbefore defined for group T and X, X′, X′′, X′′′, Y, Y′, Y′′, Y′′′ and Y′′′′ are as hereinbefore defined). Preferably each of R^{31} and R^{32} which may be the same or different (but preferably are the same) is an OC_{1-6}-alkyl group, particularly preferably OMe.

In the fourth preferred embodiment, step (A′′′0) is preferably preceded by:

(A′′′00) reacting a compound of formula XIX:

(XIX) R^{15}-CO-Y-N(R^{33})(R^{34})

(wherein Y and R^{15} are as hereinbefore defined; and each of R^{33} and R^{34} which may be the same or different is a group Y′-CO-NH-X-N-Y′′-CO-NH-X′-N-Y′′′-CO-NH-X′′-NH_2 wherein X, X′, X′′, Y′, Y′′ and Y′′′ are as hereinbefore defined) with a Michael addition reagent.

The compound of formula XIX may itself be prepared from a compound of formula XVI by step (A′′0) as hereinbefore defined.

Steps (A0), (A′0), (A′′0) and (A′′′0) may be carried out in a suitable solvent (*eg* an alcohol such as methanol) at low temperature (*eg* 0°C).

The Michael addition of steps (A00), (A′00), (A′′00) and (A′′′00) may exploit any suitable Michael addition

reagent. Preferred is an alkyl acrylate (such as a C_{1-6}-alkyl acrylate), particularly preferably methyl acrylate.

Typically the alkyl acrylate is present in acetonitrile or the corresponding alkyl alcohol (*eg* methanol for methyl acrylate).

Whilst the preferred hyperbranched amidoamine polymers according to the invention are polyamidoamines, the invention also contemplates the inclusion of further co-monomers which may add additional further functionality, stability or biological compatibility to the polymer. Such further co-monomers can include, for example, linear, i.e. un-branched monomers, such as β-alanine and derivatives thereof. Such comonomers can be present in a molar quantity of from 0 to 99%, especially from 1 to 50%, based upon the molar quantity of the AB_x monomer present.

Polymeric condensation may be induced thermally or by using an amide coupling agent. The latter has the advantage that polymeric condensation may be carried out at room temperature.

Thermal condensation is typically carried out at an elevated temperature in excess of 100°C (*eg* 200°C) and may be carried out at less than ambient pressure (*eg* under high vacuum such as at about 0.5mmHg).

Polymeric condensation may be carried out using an amide coupling agent. Numer ous amide coupling agents are known to the skilled person (see *inter alia* Handbook of Reagents for Organic Synthesis: Activating Agents and

Protecting Groups, A. J. Pearson and W. R. Roush. *John Wiley and Sons, Chichester*. 1999) and include triphenylphosphite/pyridine in N-methylpyrrolidinone (NMP) typically at a temperature in the range 40-200°C, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) in NMP typically at a temperature in the range 20-100°C or 4-(4,6-dimethoxy-1,3,5-triazin-2yl)-4-methylmorpholinium chloride (DMT-MM) in methanol or water typically at room temperature.

The product may be purified via preparative column chromatography (for high grade products) or dialysis (for general use).

The process may optionally further comprise the step of: (B1) functionally derivatising the amine groups in which the first and second irregularly branched amidoamine units terminate.

The process may optionally further comprise the step of: (B2) functionally derivatising the carboxylic acid or related group in which the third irregularly branched amidoamine unit terminates.

Suitable reagents and conditions for steps (B1) and (B2) will be familiar to those skilled in the art. For example, step (B1) can comprise rendering the terminal amine groups cationic (*eg* in aqueous solution).

Of the total number of terminal units in the hyperbranched polymers of the invention, preferably greater than 80%, more preferably greater than 90% and most preferably greater than 95% are functionalised amine

groups. Such high percentages can be obtained with the hyperbranched polymers of the invention because the terminal amine units occur throughout the polymer molecule and do not simply reside on the surface of the molecule. Preferably the hyperbranched polymer comprises less than 20% of methyl ester terminal units.

Viewed from a yet further aspect the present invention provides a composition comprising a hyperbranched amidoamine polymer as hereinbefore defined together with an agent selected from the group consisting of a therapeutically or prophylactically active agent, an *in vivo* occurring or *in vitro* generated nucleotide (*eg* a polynucleotide or oligonucleotide such as a virus or fragment thereof, expression vector, gene or fragment thereof, DNA (*eg* a single, double or multiple strand thereof) or RNA (*eg* a single, double or multiple strand thereof)), a diagnostic agent (*eg* a diagnostic contrast agent being or containing a radionuclidic, paramagnetic, superparamagnetic, ferromagnetic, ferrimagnetic, antiferromagnetic, diamagnetic, fluorescent, phosphorescent, luminescent, chemiluminescent, X-ray absorbent, UV absorbent, IR absorbent or ultrasound absorbent species), a pesticide, a toxin, a protein (*eg* an immunoglobulin such as an antibody (or fragment thereof)), an antigen, a peptide, a nucleic acid, an amino acid and a bioactive agent.

The hyperbranched amidoamine polymer may couple with, encapsulate, complex or bond to (*eg* covalently bond to) the agent. For use *in vivo*, the composition is in pharmaceutically acceptable form and where appropriate may further comprise one or more physiologically

tolerable carriers, adjuvants or excipients. Typically the composition is a solution, suspension or emulsion (*eg* an aqueous solution, suspension or emulsion).

In a preferred embodiment, the composition comprises: a hyperbranched amidoamine polymer as hereinbefore defined bound to a nucleotide or polynucleotide (such as a virus or fragment thereof, expression vector, gene or fragment thereof, DNA (*eg* a single, double or multiple strand thereof) or RNA (*eg* a single, double or multiple strand thereof)). By way of example, the DNA or RNA may be genomic DNA, mRNA, cDNA or aRNA. Particularly preferably the composition comprises: a hyperbranched polyamidoamine as hereinbefore defined bound to DNA (*eg* a single, double or multiple strand thereof).

The hyperbranched polymer may be used to transfect cells or tissues *in vitro* (eg by straightforward incubation techniques in suitable media familiar to those skilled in the art) or *in vivo* by suitable administration protocols (*eg* routes and doses).

For use as an *in vivo* transfection agent, the composition is preferably an aqueous solution of the hyperbranched amidoamine polymer. For example, the transfection agent may be a buffered aqueous solution of the hyperbranched amidoamine polymer. For example, approximately 1mg of the hyperbranched amidoamine polymers of the invention may be provided in a buffered aqueous solution of 1ml.

Viewed from a yet still further aspect the present invention provides hyperbranched amidoamine polymers (or compositions thereof) for use in therapy or prophylaxy.

Preferably the hyperbranched amidoamine polymer (or composition thereof) for use in therapy or prophylaxy in accordance with this yet still further aspect of the invention is as hereinbefore defined.

In an embodiment, the hyperbranched amidoamine polymer is used in therapy or prophylaxy as a delivery agent for a therapeutically or prophylactically active agent (*eg* drug).

In a preferred embodiment, the hyperbranched amidoamine polymer is used in gene therapy or prophylaxy. Preferably the hyperbranched amidoamine polymer is used in gene therapy or prophylaxy as a nucleotide (*eg* DNA) carrier, a transfection agent or a vector.

The hyperbranched amidoamine polymers of the invention are exceedingly versatile and may be used in numerous fields.

Viewed from an even still further aspect the present invention provides the use (*in vivo* or *in vitro*) of a hyperbranched amidoamine polymer as hereinbefore defined as a carrier, substrate or support.

The use of the hyperbranched amidoamine polymer is preferably as a nucleotide (*eg* DNA) carrier, transfection agent or vector, or as a support or substrate (*eg* a solution phase support or substrate) in combinatorial chemistry, catalysis, surface coating, implant coating and photoactive systems.

Viewed from a yet even still further aspect the present invention provides the use of a hyperbranched amidoamine polymer for the preparation of a composition (*eg* medicament) for combatting (*eg* treating or preventing) genetically related conditions or disorders.

Preferably the hyperbranched amidoamine polymer in accordance with this yet even still further aspect of the invention is as hereinbefore defined.

As novel intermediates, certain compounds of formula XI defined hereinbefore form a further patentable aspect of the invention.

Viewed from an even further aspect the present invention provides an intermediate of formula XI as hereinbefore defined.

In a first preferred embodiment of the intermediate, R^{13} and R^{14} are both the group
-Y'-CO-NH-X-NH_2.

In a second preferred embodiment of the intermediate, R^{13} and R^{14} are both the group -Y'-CO-NH-X-N-(Y''-CO-NH-X'-NH_2)$_2$.

In a third preferred embodiment of the intermediate, R^{13} and R^{14} are both the group -Y'-CO-NH-X-N-(Y''-CO-NH-X'-N(Y'''-CO-NH-X''-NH_2)$_2$)$_2$.

In a fourth preferred embodiment of the intermediate, R^{13} and R^{14} are both the group -Y'-CO-NH-X-N-(Y''-CO-NH-X'-N(Y'''-CO-NH-X''-N(Y''''-CO-NH-X'''-NH_2)$_2$)$_2$)$_2$.

The present invention will now be illustrated in a non-limitative manner with reference to the following Example and Figures 1 and 2 in which:

Figure 1 illustrates the synthetic steps for preparing AB_2 and AB_4 type monomers; and

Figure 2 illustrates results for transfection using hyperbranched polymers of the invention.

Example

The synthesis of monomers for polymerisation is initiated from a β-alanine core **1** and follows a two-step (for an AB_2 type monomer) or four-step (for an AB_4 type monomer) iterative procedure (see Figure 1). Growth of the monomer (PAMAM) units is performed by standard PAMAM synthesis described elsewhere (see for example Tomalia *et al*; Polym. J. (Tokyo), 1985, 17, 117-132).

Specific Conditions for the Synthesis of Intermediate 2

A 250ml round-bottomed flask was charged with the reagents β-alanine **1** (20g, 0.225moles), methyl acrylate (80ml, 0.9moles) and triethylamine (65ml, 0.46moles) then the mixture dissolved in anhydrous methanol (250ml). The solution was cooled to 0°C in ice and stirred under a dry atmosphere for 1 hour. The reaction was then stirred for 2 days at room temperature. After the reaction was complete the excess reagents and solvent were removed under reduced pressure to give the ester-terminated intermediate 2 as a free-flowing honey coloured oil,

yield 99%. 250MHz NMR $CDCl_3$ δ_H 2.37 (t, 2H, $C\underline{H}_2COOH$); 2.47 (t, 4H, $C\underline{H}_2CO$); 2.74 (t, 2H, $C\underline{H}_2CH_2COOH$); 2.80 (t, 4H, $NC\underline{H}_2$); 3.63 (s, 6H, $OC\underline{H}_3$); 9.11 (bs, 1H, $COO\underline{H}$). δ_C 31.5, 32.3, 48.3, 49.1, 51.2, 172.2, 175.6. IR 3410, 2955, 2844, 2622, 2490 cm^{-1} λ_{max} 1735 cm^{-1}. MS (ES^+) MH^+ 262.

Specific Conditions for the Synthesis of AB_2 type Monomer 3.

The ester-terminated intermediate **2** (53g, 0.203moles), was dissolved in 150ml anhydrous methanol and added dropwise, over a period of hour, to a stirred solution of ethylene diamine (81ml, 1.218moles) in methanol (200ml) at 0°C. After addition of the monomer was complete the reaction was stirred at room temperature under nitrogen for 7 days. Solvent and excess ethylene diamine was removed via rotary evaporation. Final traces of ethylene diamine were removed (as determined by GC and NMR) by placing the product under a high vacuum for 5 days (0.2mmHg). This gave the desired AB_2 type monomer 3 as a thick orange oil, yield 98%. 250MHz NMR d_6-DMSO δ_H 2.08(bt, 2H, $C\underline{H}_2COOH$); 2.19 (bt, 4H, $C\underline{H}_2CO$); 2.50-2.70 (bm, 10H, residual CH_2's); 3.10 (bq, 4H, $C\underline{H}_2NH$); 8.22 (bt, 2H, $N\underline{H}$). δ_C 34.4, 37.0, 40.7, 40.9, 50.8, 51.3, 173.5, 178.9. IR 3270, 3068, 2938, 2169, 1651 cm^{-1}. λ_{max} 1557 cm^{-1}. MS (FAB) MH^+ 318.

Specific Conditions for the Synthesis of Intermediate 4

The AB_2 type monomer 3 (12.158g, 3.835×10^{-2}moles in 50ml anhydrous methanol) was added dropwise to a stirred solution of methyl acrylate (21ml, 0.23moles) in methanol (50ml) over a period of 30 minutes at 0°C under a dry

atmosphere. The reaction was then stirred for 2 days at room temperature. After the reaction was complete the excess methyl acrylate and solvent were removed under reduced pressure to give the ester-terminated intermediate 4 as a thick orange oil, yield 98%. 250MHz NMR $CDCl_3$ δ_H 2.25-2.47 (m, 18H, $C\underline{H}_2N$); 2.55-2.85 (series of triplets, 14H, $C\underline{H}_2CO$); 3.15 (bq, 4H, $NHC\underline{H}_2$); 3.52 (s, 12H, $OC\underline{H}_3$); 7.02 (bt, 2H, $N\underline{H}$); 7.68 (bs, 1H, $COO\underline{H}$). δ_C 31.2, 32.1, 32.2, 32.4, 36.6, 48.7, 48.9, 51.4, 52.4, 61.9, 171.0, 172.7, 174.6. IR 3297, 2952, 2829, 2045 cm^{-1}. λ_{max} 1732 cm^{-1}. MS (FAB) MH^+ 662.

Specific Conditions for the Synthesis of AB_4-type Monomer 5

The ester-terminated intermediate **4** (23.37g, 3.536×10^{-2}moles), was dissolved in 100ml anhydrous methanol and added dropwise over an hour to a stirred solution of ethylene diamine (190ml, 2.8moles) in methanol (100ml) at 0°C. After addition of the monomer was complete the reaction was stirred at room temperature for 9 days. Solvent and excess ethylene diamine was removed via rotary evaporation. Final traces of ethylene diamine were removed (as determined by GC and NMR) by placing the product under a high vacuum for 5 days (0.2mmHg). This gave the desired AB_4 monomer 5 as a thick orange oil in quantitative yield. 250MHz NMR d_6-DMSO δ_H 2.10-2.30 (series of broad triplets, 14H, $C\underline{H}_2CO$); 2.40-2.75 (bm, 26H, residual CH_2's); 3.00-3.25 (bq, 12H, $C\underline{H}_2NH$); 8.06 (bt, 2H, $N\underline{H}$); 8.36 (bt, 4H, $N\underline{H}$). δ_C 34.6, 37.1, 38.0, 42.4, 43.3, 50.7, 51.1, 51.6, 52.2, 53.2, 172.9, 177.7. IR 3271, 3063, 2935, 2863, 2359, 2341 cm^{-1}. λ_{max} 1648 cm^{-1}. MS (FAB) MH^+ 774.

Specific Procedure for the Bulk Thermal Polymerisation of AB_2 and AB_4-type Monomers

The desired monomer was placed in a reaction tube and heated to 200°C, under high vacuum (standard laboratory pump, ~ 0.5mmHg), for 24 hours. The crude polymers were isolated as a glassy orange solids. Purification *via* membrane filtration (using a membrane bag with a 2.4nm cut-off) provided the final polymer in 40-70% yield.

Spectral data forAB$_2$-type polymer: 250MHz NMR d_6-DMSO δ_H 1.00-4.50 (series of broad multiplets, N*H*,) 1.0-2.8 (C*H*$_2$N and C*H*$_2$O H), 2.8-4.5 (C*H*$_2$NH H); 7.70-8.80 (broad singlet, N*H*). 100MHz NMR d_6-DMSO δ_C 29.3, 29.5, 31.5, 31.9, 32.6, 33.2, 33.4, 34.0, 36.5, 37.8, 38.5, 38.8, 39.5, 43.3, 43.7, 44.2, 45.7, 49.6, 49.8, 50.0, 50.3, 50.6, 51.0, 51.4, 51.8, 52.0, 52.2, 52.7, 53.0, 53.5, 54.1, 158.8, 168.2, 168.9, 171.3, 171.7, 172.5, 172.7, 173.0, 173.3, 173.4. GPC analysis (water, pH 4.5) M_w 5828, PD 2.4, (M_{z+1} 15707). TGA degradation onset 272°C, 10% wt. loss 331°C.

Specific Procedure for Polycondensation of AB_2-type Monomer using TPP/pyridine as Condensing Agent

The AB_2-type monomer (0.793g, 2.5×10^{-3} moles) was dissolved in NMP (2.5ml) with heating and then placed under a nitrogen atmosphere at 100°C. To the solution was added TPP (660µl, 2.5×10^{-3} moles) and pyridine (625µl, 7.75×10^{-3} moles) via syringe and the reaction stirred under nitrogen at 100°C for 3½h. The final orange/red reaction mixture was then quenched with methanol (20ml) and precipitated into ethyl acetate (200ml). The polymer

was isolated as a sticky yellow solid in 60% yield. 250MHz NMR d_6-DMSO δ_H 2.10-3.50 (series of broad multiplets, 58H relative to N*H*, all C*H*$_2$ protons); 8.10-8.60 (broad singlet, 8H, N*H*). 63MHz NMR d_6-DMSO δ_C 15.2, 21.9, 33.7, 34.3, 36.6, 37.1, 37.9, 38.7, 39.6, 39.8, 45.3, 50.3, 52.2, 60.9, 153.7, 153.8, GPC analysis (water, pH 4.5) M_w 3409, PD 2.6, M_{z+1} 12026. TGA degradation onset 153°C, 10% wt. loss 229°C.

Alternative Procedures for Polycondensation of AB$_2$-type and AB$_4$-type Monomers using a Condensing Agent

The AB$_n$-type monomer (1.0×10^{-3} moles) in solvent (5ml) with warming in a 3-necked round bottomed flask. Nitrogen was bubbled through the monomer solution for 15 minutes then the condensing agent(s) (1.25×10^{-3} moles) were added. The solution mixture was stirred until polymerisation was complete (as judged by GPC). The product was collected and purified *via* membrane filtration (using a membrane bag with a 2.4nm cut-off). Alternative condensing agents include triphenylphosphite/pyridine in N-methylpyrrolidinone (NMP) at various temperatures from 40-200°C or BOP (benzotriazol-1-yloxytris (dimethylamino) phosphonium hexafluorophosphate) in NMP at temperatures from 20-100°C, DMT-MM (4-(4,6-dimethoxy-1,3,5-triazin-2yl)-4-methylmorpholinium chloride) in methanol or water at room temperature.

Transfection Results

For all transfection experiments, 2µg of plasmid DNA (*lacZ*, 7.2kb) was mixed with 6µg of a an AB$_2$-type hyperbranched polyamidoamine of the invention (A) and an AB$_4$-type

hyperbranched polyamidoamine of the invention (B). These amounts resulted in complexes having a 1:3 ratio of DNA to hyperbranched polyamidoamine. The transfection efficiency against a variety of cell lines (including EAhy 926, HSVEC 1, HEK 293) was assessed using a standard β-galactosidase assay. The results for the hyperbranched polyamidoamines A and B for HEK 293 are shown in Figure 2 alongside the result for SUPERFECT^R (C), a PAMAM dendrimer with 64 terminal groups (D) and a control (E).

The reader's attention is directed to all papers and documents which are filed concurrently with or previous to this specification in connection with this application and which are open to public inspection with this specification, and the contents of all such papers and documents are incorporated herein by reference.

All of the features disclosed in this specification (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined in any combination, except combinations where at least some of such features and/or steps are mutually exclusive.

Each feature disclosed in this specification (including any accompanying claims, abstract and drawings), may be replaced by alternative features serving the same, equivalent or similar purpose, unless expressly stated otherwise. Thus, unless expressly stated otherwise, each feature disclosed is one example only of a generic series of equivalent or similar features.

The invention is not restricted to the details of any foregoing embodiments. The invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including any accompanying claims, abstract and drawings), or to any novel one, or any novel combination, of the steps of any method or process so disclosed.